

Serial No. 09/805,311
Group Art Unit: 1638

REMARKS

Reconsideration of the present application is respectfully requested. Claims 2-87 were pending in the application. Claims 2-76, and 82-87 are cancelled in the current amendment. Claims 77-81 remain under examination. Claim 81 is allowed. No new matter has been added in this amendment.

Double Patenting Rejection

Claim 2 remains rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-2 of U.S. Patent No. 6,232,527.

Claim 2 has been cancelled in the current response, thereby obviating the rejection.

Rejection under 35 U.S.C §112, 1st paragraph – Written Description

Claims 77-80 are rejected under 35 U.S.C §112, first paragraph as containing subject matter which was not described in the specification in such a way to reasonably convey to one of skill in the art that the inventor(s) had possession of the claimed invention.

The Action asserts that Appendices A and B, submitted in the paper number 7 filed 17 March 2003, do not clearly relate the evidence to the claimed percentages of sequence identity of SEQ ID NO: 2.

Applicants have modified the Multiple Sequence Alignment to note the conserved domains and residues as noted in Example 4 of the application, and the BioScout analysis (Appendix B, Paper No. 7). Applicants also submit pairwise GAP analyses comparing SEQ ID NO: 2 individually to the sequences used in the Multiple Sequence Alignment. This information is submitted as Appendix C.

Serial No. 09/805,311
Group Art Unit: 1638

As shown in the GAP analyses, SEQ ID NO: 2 shares the following overall percent identities and similarities:

SEQ ID NO: 2 vs.	Source	% Identity	% Similarity
X77041aa	<i>S. pombe</i>	51.194	63.66
L37374aa	<i>H. sapiens</i>	53.723	64.628
L26320aa	<i>M. musculus</i>	54.301	65.323

The present invention describes Rad2/FEN-1 endonucleases having approximately 51% - 55% sequence identity to other known Rad2/FEN-1 endonucleases, and further comprising conserved amino acids and domains common to Rad2/FEN-1 polypeptides. Using a standard technique of Multiple Sequence Alignment (e.g. Appendix C), one of skill in the art could discover the evolutionary tolerance for amino acid substitutions, insertions or deletions (*i.e.* gaps) at given positions in the polypeptide.

The Multiple Sequence Alignment presented in Appendix C, which could have been done by one of skill in the art at the time of filing, shows several regions conserved in Rad2/FEN-1 polypeptides, and also suggests locations and amino acid substitutions likely to be tolerated in both conserved and non-conserved domains. The role of certain amino acids were demonstrated by mutation to alanine as indicated in Example 4 and Appendix C. Appendix C shows individual and blocks of amino acids which are identical to the corresponding amino acid of SEQ ID NO: 2, or similar (conservative substitutions), or not conserved.

For example, as expected the pfam|hmm|XPG_N domain from amino acid 1 – 108 (SEQ ID NO: 2 numbering) shows a high degree of sequence identity, including several conserved amino acids whose functional relevance was noted by mutational analysis in Example 4. It is also noteworthy that not all amino acids in this domain are absolutely conserved, for example, there is a lysine at position 4 of SEQ ID NO: 2 and X77041, however non-conservative amino acids Histidine and Glutamine are

Serial No. 09/805,311
Group Art Unit: 1638

found in L26320 and L37374 respectively, indicating the likelihood that the polypeptide function (flap endonuclease) would be maintained even when lysine is substituted for at this position. At amino acid 9, X77041 comprises a Valine, which is a conservative substitution relative to Leucine, which is found at that position in the other sequences compared. This indicates that a conservative substitution (e.g. Valine, Isoleucine, or Methionine) for Leucine is most likely tolerated at this position, maintaining flap endonuclease activity.

The present invention describes Rad2/FEN-1 endonucleases having approximately 51% - 55% sequence identity to other known Rad2/FEN-1 endonucleases, and further comprising conserved amino acids and domains common to Rad2/FEN-1 polypeptides. Using a standard technique of Multiple Sequence Alignment (e.g. Appendix C), one of skill in the art could discover the evolutionary tolerance for amino acid substitutions, insertions or deletions (i.e. gaps) at given positions in the polypeptide, and further predict the likely impact of other modifications.

Therefore, Applicants submit that the specification, coupled with the state of the art at the time of filing reasonably conveys to one of skill in the art that the inventor had possession of polypeptides having 80, 85, 90, or 95% sequence identity to SEQ ID NO: 2 and having flap endonuclease activity as recited in Claims 77-80. Applicants respectfully request that the rejection of Claims 77-80 under 35 U.S.C §112, first paragraph be withdrawn.

Rejection under 35 U.S.C §101 – Utility

Claims 3, 75, 82 and 83 are rejected under 35 U.S.C §101 because the claimed invention is directed to non-statutory subject matter. The action asserts these claims read on a maize cell found in nature.

Claims 3, 75, 82, and 83 have been cancelled in the current response, thereby obviating the rejection under 35 U.S.C §101.

Serial No. 09/805,311
Group Art Unit: 1638

Rejection under 35 U.S.C §102, Anticipation

Claims 3-6, 75, 76 and 82-87 are rejected under 35 U.S.C. § 102(b) as being anticipated by Baszczynski *et al.* US 5,824,870 (issued Oct. 20, 1998).

The action asserts that Baszczynski *et al.* teaches a nonhuman host cell, a transgenic maize plant, comprising a polypeptide having 80, 85, 90, and 95% identity with SEQ ID NO: 2, thereby anticipating the claimed invention.

Claims 3-6, 75, 76 and 82-87 have been cancelled in the current response, thereby obviating the rejection 35 U.S.C. § 102(b) based on Baszczynski *et al.*, US 5,824,870.

However, for the record Applicants address the rejection as it may be applied to pending claims 77-81, directed to polypeptides having 80, 85, 90 or 95% sequence identity with SEQ ID NO: 2. Baszczynski *et al.*, US 5,824,870 teaches the commercial production of aprotinin in plants, and discloses one polynucleotide sequence (SEQ ID NO: 1, GenBank AR050162) of 276 nucleotides in length, which encodes a barley alpha amylase signal sequence fused to the mature aprotinin protein sequence. US 5,824,870 does not disclose any amino acid sequence, particularly it does not disclose any amino acid sequence having 80, 85, 90 or 95% sequence identity with SEQ ID NO: 2 which encodes a polypeptide having flap endonuclease activity as claimed in claims 77-80 of the application, or the polypeptide of SEQ ID NO: 2 in claim 81. Therefore, Baszczynski *et al.*, US 5,824,870 does not anticipate claims 77-81, which may have been inferred from the statement of the rejection of claims 3-6, 75, 76 and 82-87 under 35 U.S.C. § 102(b).

Rejection under 35 U.S.C §112, second paragraph

Claims 4-6, 76, and 84-87 are rejected under 35 U.S.C §112, second paragraph as being indefinite.

The Action asserts that the claims are indefinite in that the relationship between "transgenic" and the recitation of the claimed "polypeptide" is unclear.

Serial No. 09/805,311
Group Art Unit: 1638

Claims 4-6, 76, and 84-87 have been cancelled, thereby obviating the rejection under 35 U.S.C §112, second paragraph.

**RECEIVED
CENTRAL FAX CENTER**

SEP 03 2003

Serial No. 09/805,311
Group Art Unit: 1638

OFFICIAL

CONCLUSION

In light of the foregoing remarks and amendments, it is believed the claims are in condition for allowance. Entry of the amendment and withdrawal of the outstanding rejections and allowance of all of the remaining claims is respectfully requested. The Examiner is invited to telephone the Applicant's representative in order to expedite prosecution and allowance of the current application.

Respectfully submitted,



Virginia Dress
Agent for Applicant(s)
Registration No. 48,243

PIONEER HI-BRED INTERNATIONAL, INC.
Corporate Intellectual Property
7100 N.W. 62nd Avenue
P.O. Box 1000
Johnston, Iowa 50131-1000
Phone: (515) 270-4192
Facsimile: (515) 334-6883

```
0961D APPENDIX C (modified version of Appendix A submitted 3/17/03)
CCG FileUp of: @/tmp/45742915.list
```

Symbol comparison table: genrundata:blosum62.cmp CompCheck: 1102

GapWeight: 8 GapLengthWeight: 2

```
0961L26320aa_pileup_45742.txt MSF: 383 Type: F March 6, 2003 13:14 Check:
3748 ..
```

L26320aa Mouse FEN-1 (Rad2) protein encoded by GenBank L26320
L37374aa Human FEN-1 (Rad2) protein encoded by GenBank L37374
X77041aa S. pombe Rad2 protein encoded by GenBank X77041
0961sid2 0961D SEO ID NO: 2

IDENTICAL TO G961962;

* , # , ! show known mutants described in Example 4.

* - Mutation of amino acid to Alanine does not affect substrate binding, but results in loss of catalytic activity (D34, D86, or D181)

- Mutation of amino acid to Alanine affected neither binding nor catalysis (R103, D179) Applicants note that SEQ ID NO: 2 has a conservative substitution of Lysine (K) at position 103, since Alanine at this position did not affect activity, a Lysine substitution is also likely active.

! - Mutation of amino acid to Alanine caused loss of binding and catalysis (G231, or D233)

As shown in Appendix B submitted in Paper No. 7, SEQ ID NO: 2 possesses two conserved XPG domains. These are noted as pfam|XPG_N and pfam|XPG_I and underlined or double underlined respectively.

[illegible]

51 * 100 *D86A mutant
 L26320aa GVLQNEEGE TTSILMGMY RTIRLNGE KPVVVDGKE EQKSGELAK
 L37374aa GVLQNEEGE TTSILMGMY RTIRLNGE KPVVVDGKE EQKSGELAK
 X77041aa GQQLNEEGE TTSILMGMY RTIRLNGE KPVVVDGKE EQKSGELAK
 0961sid2 MATHNEAGE VTSHIQMEN RTIRLNGE KPVVVDGKE EQKSGELAK

pfam|XPG_N
(aa 1-108)

101# 150 #R103A mutant

L26320aa RSEFRAAEK QIQQAQAGM EEEERERKR LKRVYTHN GHHHETSMG
L37374aa RSEFRAAEK QIQQAQAGM EQEAEERKR LKRVYTHN GHHHETSMG
X77041aa RVEHQKARE IQFEKAVGT AEMAEERKR LKRVYTHN AKRLIELMG
0961sid2 RYARDATK DTEAVEVGD KDAERLKG TVKVTROHN GKRLIELMG

151 ! # * 200 !E156A mutant
L26320aa EYAPSEEA EASGAALAKA GKVAAASD NDCTPPG RV LRPHITASE #D179A mutant
L37374aa EYAPSEEA EPGGAALVKA GKVAAASD NDCTPPG RV LRPHITASE *D181A mutant
X77041aa PFYNLECEA EAGCAALARS GKVAASD ND LCFQAPV LRPHITFSEQ pfam|XPG_I
0961sid2 EYVAPSEEA EALCAALCIN DKVAVASED ND LCTFGAPR FLRHMDPS (aa 147-238)

```

201                                     ! !                               250
L26320aa KKEPQEDH VLOEGEN Q Q EHGEL LGSDYC ST GYAKRA DE !G231A mutant
L37374aa KKEPQEDH VLOEGEN Q Q EHGEL LGSDYC ST GIGPKRA DE !D233A mutant
X77041aa KKEPSEIN EHALNGH Q Q EHGEL LGSDYC PL GGPARA ET
0961sid2 KKEPMEED VVHEELQ Q Q EHGEL CGSDYC ST GIGGQTA KL

251                                     300
L26320aa TQKHKSTEE RRDPSPYP PENWLHKEA QQLLEEEV DP SVELKW
L37374aa TQKHKSTEE RRDPNPYP PENWLHKEA HQELEEEV DP SVELKW
X77041aa TROYGELRF KEADSPYP PEDWPEA RRLLEAEV PG EIEELNK
0961sid2 TROHGSTST ENINDEYQ TPEDWYQEA RRLKEENV .L IPELKW

301                                     350
L26320aa EPNEELKE CGKQFSE RRSCKKS KSGSGR LDPVTEG
L37374aa EPNEELKE CGKQFSE RRSCKKS KSGSGR LDPKVTG
X77041aa EPDASIQK KQENED RVKLGN E KSKTIQGR LSPKEVP
0961sid2 EPDEGLST LVKNGENED RVKATEK SAKKSGR LSEKETA

351                                     383
L26320aa LS.SAKKKEP EPKGP.AKKK AKGGACKF RGK
L37374aa LS.SAKKKEP EPKGS.AKKK AKGGACKF RGK
X77041aa .S.EKRPV KSKGS.AKKK DNKGGSK KKR
0961sid2 TSAELKKRT SSKTSKAAAN KTKAGCKK ~~~

```


GAP of: 0961X77041aa check: 1477 from: 1 to: 380

WPDEF Case 0961 Rad2 protein encoded by GenBank X77041 S pombe

Case 0961 Rad2 protein encoded by GenBank X77041 S pombe

X77041. Saccharomyces pom. . . [gi:2909339] Links

LOCUS SPRAD2 1143 bp DNA linear PLN 25-FEB-1999

DEFINITION Saccharomyces pombe rad2 gene.

ACCESSION X77041 REGION: join(434..446,516..606,988..2026) . . .

to: 0961sid2 check: 985 from: 1 to: 379

WPDEF Case 0961 Rad2/FEN-1 SEQ ID NO: 2 corn

Case 0961 Rad2/FEN-1 SEQ ID NO: 2 corn

Symbol comparison table: blosum62.cmp CompCheck: 1102

BLOSUM62 amino acid substitution matrix.

Reference: Henikoff, S. and Henikoff, J. G. (1992). Amino acid substitution matrices from protein blocks. Proc. Natl. Acad.

Sci. USA 89: 10915-10919.

Gap Weight: 8 Average Match: 2.778
Length Weight: 2 Average Mismatch: -2.248

Quality: 1019 Length: 382
Ratio: 2.689 Gaps: 2
Percent Similarity: 63.660 Percent Identity: 51.194

Match display thresholds for the alignment(s):

| = IDENTITY
: = 2
. = 1

0961X77041aa x 0961sid2 September 2, 2003 17:11 ..

```

1 MGIKGLAQVLSEHAPASVKHNDIKNYFGRKVAIDASMSLYQFLIQVRSQD 50
| | | | | . . . . . | . . . . . | | | | | : : | | | | | : | | | | |
1 MGIKGLTKLLADNAPKAMKQKQFESYFGRKIAVDASMSIYQFLIVVGRTG 50

51 GQQLMNEQGETTSHLMGMFYRTLRIVDNGIKPCFVFDGKPPPTLKSGELAK 100
: | | | | | | | | | | : : : : | | | | | : | | | | |
51 METLTNEAGEVTSHLQGMFNRTIRLLEAGIKPVYVFDGKPPDMKKQELAK 100

101 RVARHQKAREDQEETKEVGTAEMVDRFAKRTVKVTRQHNDKAKRLLELMG 150
| . : | . | | | | : : : - | | | | | | | | : : | | | | |
101 RYSKRDDATKDLTEAVEVGDKDAIEKLSKRTVKVTRQHNDCKRLLRLMG 150

151 IPFVNAPCEAEQAALARSGKVYAAASEDMDTLCFQAPVLLRHLTFSEQ 200
: | | | | | : | | | | . | : | | | | | . | | | | |
151 VPVVEAPSEAEAECAALCINDKVFVASEDMDSLTFGAPRFLRHLMDPSS 200

201 RKEPISEYNIKALNGLDMSVEQFVDLCILLGCDYCEPIRGVGPAREVEL 250
: | | : : : | | | : : : : | | | | | | | | : : : | . . |

```

201 KKIPVMEFDVAKVLESELTMDQFIDLCLCGCDYCDSEIKGIGGQTALKL 250
251 IRQYGTLDRLFVKEADRSKYPIPEDWPFYEDARRLPLDAEVLPGESIIEKWK 300
|||:|.:. . . .:| |||||:|:| ||| : | : |||
251 IRQHGSIESILENLNKDORYQIPEDWPFYQEARRLFKEPNVTL.DIPELKWT 299
301 SPDADGIIQFLVKEKGFNEDRVKLGINRLEKASKTIPQGRIDSFFKP..V 348
|.|| :|:| |||: ||||| | :. | |||:| |||
300 APDEEGLISFLVKDNGFNEDRVTKAIEKIKSAKNKSSQGRLESFFKPTAT 349
349 PSSPKKPVDTKSKGSAKRKRDSNKGESKKKR 380
|.|| :| | | | | | |
350 TSAPLKRKETSDKTSKAAANKKTKAGGKKK.. 379

GAP of: 0961sid2 check: 985 from: 1 to: 379

WPDEF Case 0961 Rad2/FEN-1 SEQ ID NO: 2 corn

Case 0961 Rad2/FEN-1 SEQ ID NO: 2 corn

to: 0961sid10 check: 3392 from: 1 to: 380

WPDEF Case 0961 Rad2/FEN-1 SEQ ID NO: 10 HUMAN

Case 0961 Rad2/FEN-1 SEQ ID NO: 10 HUMAN (GB L37374aa)

Used in sequence comparison, showing of conserved domains in Table 1

Symbol comparison table: /app/gcg/10.2/gcgcore/data/rundata/blosum62.cmp

CompCheck: 1102

BLOSUM62 amino acid substitution matrix.

Reference: Henikoff, S. and Henikoff, J. G. (1992). Amino acid substitution matrices from protein blocks. Proc. Natl. Acad. Sci. USA 89: 10915-10919.

Gap Weight: 8 Average Match: 2.778
Length Weight: 2 Average Mismatch: -2.248

Quality: 1031 Length: 383
Ratio: 2.720 Gaps: 4
Percent Similarity: 64.628 Percent Identity: 53.723

Match display thresholds for the alignment(s):

| = IDENTITY
: = 2
. = 1

0961sid2 x 0961sid10 March 3, 2003 13:34 ..

```

1 MGIKGLTKLIAADNAPKAMKEQKFESYFGRKIAVDASMSIYQFLIVVGRG 50
  |||.|| ||:| | | |.:| .|||||:|:||||||| | | |
1 MGIQGLAKLIADVAPSAIRENDIKSYFGRKVAIDASMSIYQFLIAV.RQG 49

51 METLTNEAGEVTSHLQGMFNRTIRLLEAGIKPVYVFDGKPPDMKKQELAK 100
  : | | | | ||| | | | | | | | | | | | | | | | | | |
50 GDVLQNEEGETTSHLMGMFYRTIRMMENGIKPVYVFDGKPPQLKSGELAK 99

101 RYSKRDDATKDLTEAVEVGDKDAIEKLSKRTVKVTRQHNEDCKRLLRLMG 150
  | :| :| | | :| | . :| | | | | | | | | | | | |
100 RSERRAEAEKQLQQAQAAGAEQEVEKFTKRLVKVTKQHNDECKRLLSLMG 149

151 VPVVEAPSEAEAECAALCINDKVFVASEDMDSITFGAPRFLRHLMDFSS 200
  : | .:||||| | | | | | | | | | | | | | | | | | |
150 IPYLDAPSEAEASCAALVKAGKVYAAATEDMDCLTFGSEVLMRHLTASEA 199

201 KKIPVMEFDVAKVLEELTMDQFIDLCLCGCDYCDYCDYCDYCDYCDYCDY 250
  ||:| | | .:|:| | | :|:| | | | | | | | | | | | | |
200 KKLPIQEFHLSRILQELGLNQEQFVDLCILLGSDYCESIRGIGPKRAVDL 249

251 IRQHGSIESILENLNKORYQIPEDWPYQEARRLFKEPNV.TLDIPELKWT 299
  |..| ||| | . | .:| :| | | :. | | | | | | | | | |
250 IQKHKSIEEIVRRIDPNKYVPENWLHKEAHQLFLEPEVLDPESEVELKWS 299

```

300 APDEEGLISFLVKONGFNEDRVTKAIEKIKSAKNKSSQGRLESFFKPTAT 349
|.|| || | : |.||:| :.:| :|.||||: ||| |.
300 EPNEEELIKFMCGEKQFSEERIRSGVKRLSKSRQGSTQGRLODDFFKVTGS 349
350 TSAPLKRKETSDKTSKAAANKKTKAGGKKK... 379
|. |||| | | || | || |
350 LSS.AKRKEPEPKGS.TKKKAKTGAAGKFKRGK 380

GAP of: 0961sid2 check: 985 from: 1 to: 379

WPDEF Case 0961 Rad2/FEN-1 SEQ ID NO: 2 corn

Case 0961 Rad2/FEN-1 SEQ ID NO: 2 corn

to: 0961L26320aa check: 8435 from: 1 to: 378

WPDEF Case 0961 Rad2/FEN-1 protein encoded by GenBank L26320 mouse

Case 0961 Rad2/FEN-1 protein encoded by GenBank L26320 mouse

L26320. Mouse flap endonu. . .[gi:499648] Links

LOCUS MUSFEN1X 2033 bp mRNA linear ROD 28-SEP-1994

DEFINITION Mouse flap endonuclease-1 (FEN-1) mRNA, complete cds.

ACCESSION L26320 . . .

Symbol comparison table: /app/gcg/10.2/gcgcore/data/rundata/blosum62.cmp

CompCheck: 1102

BLOSUM62 amino acid substitution matrix.

Reference: Henikoff, S. and Henikoff, J. G. (1992). Amino acid substitution matrices from protein blocks. Proc. Natl. Acad. Sci. USA 89: 10915-10919.

Gap Weight: 8 Average Match: 2.778
Length Weight: 2 Average Mismatch: -2.248

Quality: 1014 Length: 385
Ratio: 2.683 Gaps: 6
Percent Similarity: 65.323 Percent Identity: 54.301

Match display thresholds for the alignment(s):

| = IDENTITY
: = 2
. = 1

0961sid2 x 0961L26320aa March 3, 2003 14:07 ..

```

1 MGIKGLTKLLADNAPKAMKEQKFESYFGRKIAVDASMSIYQFLIVVGRIG 50
  ||| || ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
1 MGIHGLAKLIADVAPSAIRENDIKSYFGRKVAIDASMSIYQFLIAV.RQG 49

51 METLTNEAGEVTSHLQGMFNRTIRLLEAGIKPVYVFDGKPPDMKKQELAK 100
  : ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
50 GDVLQNEEGETTS.LMGMFYRTIR.MENGIKPVYVFDGKPPQLKSGELAK 97

101 RYSKRDDATKDLTEAVEVGDKDAIEKLSKRTVKVTRQHNEDCKRLLRLMG 150
  | :| :| :| :| :| :| :| :| :| :| :| :| :| :| :| :|
98 RSERRAEAEKQLQQAQEAGMEEEVEKFTKRLVKVTKQHNDECKHLLSLMG 147

151 VPVVEAPSEAEAECAALCINDKVFVASEOMDSLTFGAPRFLRHLMDPSS 200
  :| :| :| :| :| :| :| :| :| :| :| :| :| :| :| :|
148 IPYLDAPSEAEASCAALAKAGKVYAAATEDMDCLTFGSPVLMRHLTASEA 197

201 KKIPVMEFDDVAKVLEELELTMDQFIDLCLCGCDYCDYCDYCDYCDYCDY 250
  ||| :| :| :| :| :| :| :| :| :| :| :| :| :| :| :|
198 KKLPIQEFHLSRVLQELGLNQEQFVDLCILLGSDYCDYCDYCDYCDYCDY 247

```

```

251 IRQHGSIESILENLNKDRYQIPEDWPYQEARRLFKEPNVT.LDIPELKWT 299
   |..| | | | | . | . | : | | . | : | | . | | | | : | | | .
248 IQKHKSTEEIVRRLDPSKYVPENWLHKEAQQLFLEPEVVDPESEVELKWS 297
   . . . . .
300 APDEEGLISFLVKDNGFNEDRVTKAIEKIKSAKNKSSQCRLESFFKPTAT 349
   |..| | : | : : |..| : : : : : : : |..| | : | | | | .
298 EPNEEELVKFMCGEKQFSEERIRSGVKRLSKSRQGSTQGRLODFEKVTGS 347
   . . . . .
350 TSAPLKRKETS DKT SKAAANKKTKAGGKKK..... 379
   | . | | | | | | | | | | | | | | | | | | | | | | | | | | |
348 LSS.AKRKEPE...PKGPAKKKAKTGGAGKFRRGK 378

```